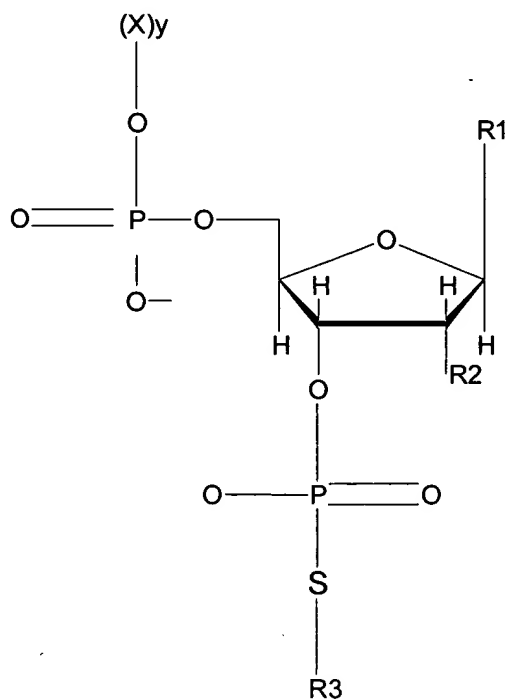


CLAIMS LISTING

The following listing of the claims replaces all previous versions.

1. (Currently Amended) A method of non-enzymatic ligation of a nucleic acid, comprising contacting a first polynucleotide comprising a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide under conditions that allow nucleophilic attack by a 5'-OH group of the acceptor polynucleotide on the polynucleotide-3' phosphorothiolate to form ~~formation of a phosphodiester bond between said first polynucleotide -3' phosphorothiolate and~~ said acceptor polynucleotide, wherein a phosphodiester bond is formed between said first polynucleotide ~~-3' phosphorothiolate~~ and said acceptor polynucleotide, whereby a ligated nucleic acid product is generated.

2. (Currently Amended) The method of claim 1, wherein said first polynucleotide comprising a polynucleotide-3' phosphorothiolate comprises a moiety having the formula:



wherein,

X is a nucleotide;

y is a positive integer;

R1 is a nucleotide base;

R2 is a hydrogen atom or hydroxyl; and

R3 is nitrophenyl.

3. (Currently Amended) The method of claim 1, wherein said first polynucleotide comprising a polynucleotide-3' phosphorothiolate further comprises a duplex polynucleotide.

4. (Previously Presented) The method of claim 1, wherein said acceptor polynucleotide further comprises a duplex polynucleotide.

5. (Currently Amended) A method of replicating a ligated nucleic acid product, comprising:

(a) contacting a first polynucleotide comprising a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide under conditions that allow nucleophilic attack by a 5'-OH group of the acceptor polynucleotide on the polynucleotide-3' phosphorothiolate to form ~~formation of~~ a phosphodiester bond between said first polynucleotide ~~-3' phosphorothiolate~~ and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said first polynucleotide-3' phosphorothiolate and said acceptor polynucleotide to generate a ligated nucleic acid product, wherein one of said first polynucleotide-3' phosphorothiolate or said acceptor polynucleotide comprises a vector, and

(b) transducing into a host cell said ~~polynucleotide~~ ligated nucleic acid product, wherein said polynucleotide product is replicated in said host cell.

6. (Currently Amended) A method of non-enzymatic ligation of a nucleic acid, comprising:

(a) contacting a polynucleotide-3' phosphorothiolate precursor and an activator under conditions sufficient to react said polynucleotide-3' phosphorothiolate precursor and said activator, wherein said polynucleotide-3' phosphorothiolate precursor reacts with said ~~iodonitrobenzene~~ activator to produce a ~~polynucleotide-3' phosphorothiolate~~ an intermediate polynucleotide comprising a polynucleotide-3' phosphorothiolate, and

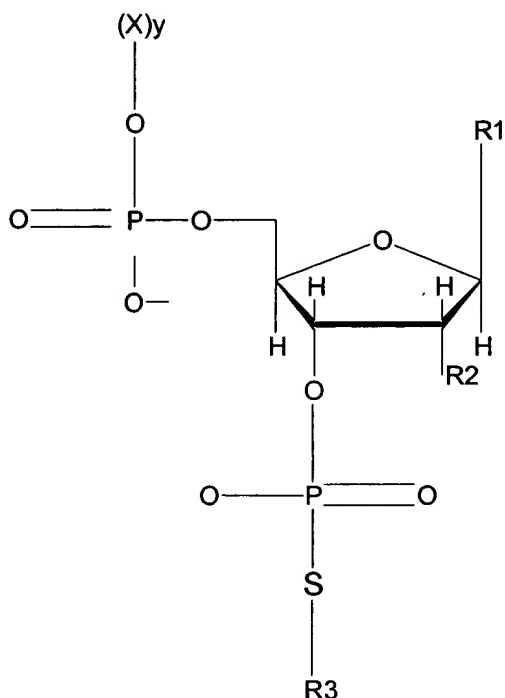
(b) contacting said intermediate polynucleotide-3' phosphorothiolate with an acceptor polynucleotide under conditions that allow nucleophilic attack by a 5'-OH group of the acceptor polynucleotide on said polynucleotide-3' phosphorothiolate to form ~~formation of a~~ phosphodiester bond between said intermediate polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said intermediate polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, whereby a ligated nucleic acid product is generated.

7. (Previously Presented) The method of claim 6, wherein said activator is iodonitrobenzene.

8. (Currently Amended) A method of ligating a vector and an insert comprising, contacting an insert comprising a polynucleotide-3' phosphorothiolate with an acceptor vector under conditions that allow nucleophilic attack by a 5'-OH group of the acceptor vector on the polynucleotide-3' phosphorothiolate of the insert to form ~~formation of a~~ phosphodiester bond between said insert and said acceptor vector, wherein a phosphodiester bond is formed between said insert and said acceptor vector, whereby a ligated product vector comprising said insert is generated.

9. (Previously Presented) The method of claim 8, further comprising transforming said vector comprising said insert into a host cell.

10. (Currently Amended) The method of claim 8, wherein said polynucleotide-3' phosphorothiolate comprises a moiety having the formula:



wherein,

X is a nucleotide;

y is a positive integer;

R₁ is a nucleotide base;

R₂ is a hydrogen atom or hydroxyl; and

R₃ is nitrophenyl.

11. (Previously Presented) A method of ligating a vector and an insert comprising:

(a) contacting a polynucleotide-3' phosphorothiolate precursor and iodonitrobenzene under conditions sufficient to react said polynucleotide-3' phosphorothiolate precursor and said

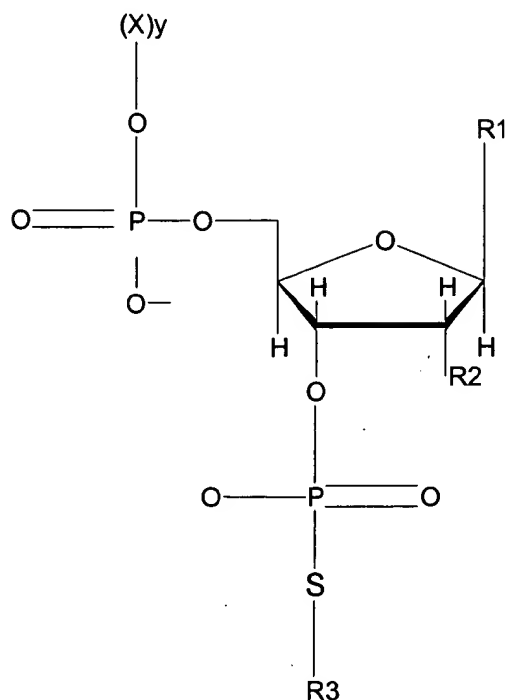
iodonitrobenzene, wherein said polynucleotide-3' phosphorothiolate precursor reacts with said iodonitrobenzene to produce a polynucleotide-3' phosphorothiolate, and

(b) contacting an insert comprising said polynucleotide-3' phosphorothiolate with an acceptor vector under conditions that allow formation of a phosphodiester bond between said insert and said acceptor vector, wherein a phosphodiester bond is formed between said insert and said acceptor vector, whereby a ligated product vector comprising said insert is generated.

12. (Currently Amended) A method of ligating a vector and an acceptor polynucleotide comprising, contacting a vector comprising a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide, under conditions that allow nucleophilic attack by a 5'-OH group of the acceptor polynucleotide on the 3'-phosphorothiolate of the vector to form ~~formation of~~ a phosphodiester bond between said vector and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said vector and said acceptor polynucleotide, whereby a ligated product vector comprising said acceptor polynucleotide is generated.

13. (Previously Presented) The method of claim 12, further comprising transforming said vector comprising said acceptor polynucleotide into a host cell.

14. (Previously Presented) The method of claim 12, wherein said polynucleotide-3' phosphorothiolate comprises a moiety having the formula:



wherein,

X is a nucleotide;

y is a positive integer;

R1 is a nucleotide base;

R2 is a hydrogen atom or hydroxyl; and

R3 is nitrophenyl.

15. (Previously Presented) The method of claim 12, wherein said vector further comprises a 3' phosphorothiolate moiety at one or more terminal ends of said vector.

16. (Currently Amended) A method of ligating a vector and an acceptor polynucleotide comprising:

(a) contacting a vector comprising a polynucleotide-3' phosphorothiolate precursor ~~and~~ with an activator under conditions sufficient to react said polynucleotide-3' phosphorothiolate precursor and said activator to produce a polynucleotide-3' phosphorothiolate, wherein said

polynucleotide-3' phosphorothiolate precursor reacts with said ~~iodonitrobenzene~~ activator to produce a polynucleotide-3' phosphorothiolate, and

(b) contacting ~~[[a]]~~ said vector comprising said polynucleotide-3' phosphorothiolate with an acceptor polynucleotide, under conditions that allow nucleophilic attack by a 5'-OH group of the acceptor polynucleotide on the polynucleotide-3' phosphorothiolate to form ~~formation of~~ a phosphodiester bond between said vector and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said vector and said acceptor polynucleotide, whereby a ligated product vector comprising said acceptor polynucleotide is generated.

Claims 17-56 (Canceled)